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GENETIC RECOMBINATION IN PSEUDOMONAS AERUGINOSA

In view of the unusual nature of the genetic processes that occur in bacteria it is desirable that a range of bacteria be studied in order to determine the generality of the mechanisms. The techniques developed by Lederberg for Escherichia coli are applicable to certain other bacteria and have proved successful with Pseudomonas aeruginosa, a gram negative bacillus characterized by the formation of blue and green pigments.

P. aeruginosa will grow on a medium consisting of glucose and mineral salts. Auxotrophic mutants for a range of amino acids, purines and pyrimidines were isolated using manganous chloride as the mutagen. In certain cases between different auxotrophic mutants resulted in the formation of recombinant prototrophs. The interfertility of the four strains used is shown in Fig. 1

FIG. 1 THE FORMATION OF PROTOTROPHS IN CROSSES BETWEEN AUXOTROPHS IN FOUR STRAINS OF PSEUDOMONAS AERUGINOSA.

+ = recombination ± = recombination at low frequency
- = no recombination

	I	L	3	29
I	-	+	+	+
L		±	-	±
3			-	-
29				-

Different mutant combinations of any two fertile parents gave different recombination frequencies of prototrophs (for example see Table 1)

Table 1 over

TABLE 1 RECOMBINATION FREQUENCIES OF I (LIL⁻) WITH VARIOUS AUXOTROPHS OF STRAIN L.

<u>Cross</u>	<u>Recombinants/10⁹ total parental cells</u>
I (LIL ⁻) x L (T ₁ ⁻)	20
x L (Ad ⁻)	55
x L (Thr ⁻)	<1
x L (IV ⁻)	>200

I (LIL⁻) auxotroph of strain I requiring leucine

T, tryptophane, Ad - adrine, Thr - threonine, IV - isoleucine and valine

Segregation of various non-selective markers has also been shown and the frequent formation of novel combinations of non-selective markers provides conclusive evidence of some genetic mechanism.

For example, in the cross I (T₁-IV₁⁺S^{SC}R^r) - a tryptophane requiring streptomycin sensitive chloramphenicol resistant mutant of strain I - x L (IV₁-T₁⁺S^{RC}S) - an isoleucine plus valine requiring streptomycin resistant chloramphenicol sensitive mutant of strain L - the formation of recombinants which are genotypically T⁺ IV⁺ S^{RC}R^r can only be explained by genetic reassortment of the marker genes.

There is considerable evidence that the mechanism of recombination is very similar to that occurring in E. coli. In both organisms actual contact of the parental bacteria is necessary for genetic recombination to take place. There is a correlation between the F fertility system in E. coli and the interfertility of the various strains shown in Table 1. For the combination I x L, with which most work has been done, equation of strain I as the F⁻ and strain L as the F⁺ gives a situation identical with that of E. coli. In both organisms there is an unequal contribution of the parents

to the recombinant, the F^- parent contributing more than the F^+ . Thus the recombinants tend to resemble more the F^- parent. Strain I and Strain L differ in their pigment forming ability, colonial morphology and in their reactions to a group of bacteriophages. 99% of recombinants resemble the strain I parent in these characteristics. Irradiation with ultra-violet light of the F^- strain of both organisms increases recombination frequency, whereas no such effect occurs with the F^- strain. In *E. coli* the F factor is infectious being easily transferred from a F^+ to a F^- strain. Infectious transfer of this nature has been shown for *Pseudomonas* in preliminary experiments at low frequency. The paucity of suitable markers in *P. aeruginosa* has limited the linkage studies. It has been possible to divide the available markers into two groups, those which are inherited freely from the F^+ parent by the F^- parent and those which fail to be inherited in this fashion. On the assumption of linearity it has been possible to establish a provisional chromosome map of the former group. Little is known yet of the actual processes of conjugation and genetic transfer in *Pseudomonas*. Such data as is available is in agreement with the process of genetic transfer recently advanced by Wollman, Jacob and Hayes for *E. coli*. They have suggested that only a part of the donor (or F^+) chromosome is transferred to the recipient or F^- cell to form an incomplete zygote.

Current work with *P. aeruginosa* is aimed at learning more of the actual mechanism of genetic transfer and also the manner of the inheritance of prophages from a multi-lysogenic strain which has been derived from strain L.